AMENDMENT UNDER 37 C.F.R. § 1.111 Attorney Docket No.: Q86534

U.S. Patent Application No.: 10/525,717

AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph bridging pages 9 and 10 with the following amended

paragraph:

In order to isolate the objective compound of the present invention from the culture,

techniques usually used for extraction and purification of metabolites produced by

microorganisms can be appropriately employed. For example, the objective compound among

compounds in the culture is extracted by adding an organic solvent such as ethyl acetate which

does not mix with water directly to the culture or to a culture obtained by centrifugation or by

filtration after adding a filter aid to the culture mixture. The objective compound can also be

extracted by allowing the culture to contact with an appropriate carrier, thereby effecting

adsorption of the produced compound in the filtrate to the carrier, and then eluting the compound

with an appropriate solvent. For example, the compound is adsorbed by allowing it to contact

with a porous adsorption resin such as Amberlite Amberlite™ (trade name) XAD-2, Diaion

<u>Diaion[™]</u> (trade name, hereinafter same as above) HP-20, Diaion <u>Diaion</u> CHP-20 or Diaion <u>Diaion</u>

DiaionTM SP-900. Next, the compound is eluted using an organic solvent such as methanol,

ethanol, acetone, butanol, acetonitrile or chloroform, alone or as a mixture, or a mixed solution

of the solvent with water. In some cases, a fraction containing the compound can be efficiently

obtained by increasing the mixing ratio of the organic solvent from a low concentration to a high

concentration stepwise or continuously. When extracted with an organic solvent such as ethyl

acetate or chloroform, the compound is extracted by adding such solvent to the culture filtrate

and thoroughly shaking the mixture. Thereafter, the fraction containing the compound thus

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obtained using each of the above procedures can be separated and purified with higher purity by using a usually used method such as a column chromatography which uses silica gel, ODS or the like, a centrifugal liquid-liquid partition chromatography or a high performance liquid chromatography (HPLC) which uses ODS or recrystallization.

Please replace the paragraph bridging pages 17 and 18 with the following amended paragraph:

After adjusting 200 L of the thus obtained culture with sulfuric acid to be pH 3.0, the culture was separated into cells and supernatant by a Sharples centrifuge. The supernatant was allowed to be passed through a column which has outer diameter of 18 cm and height of 150 cm packed with 20 L of DiaionDiaionTM, HP-20 (Mitsubishi Chemical Co.) and the objective compound and the like were adsorbed thereto. Subsequently, the column was washed with 50 L of tap water, then washed with 40 L of 30% methanol/water, followed by 100 L of 30% acetone/water, and finally the objective compound was eluted with 60 L of methanol. To the eluted solution, 5 L of distilled water was added and concentrated under a reduced pressure to remove methanol. An equal amount of ethyl acetate was added thereto, and ethyl acetate extraction was performed at pH 3.0 for three times. After carrying out dehydration by adding anhydrous sodium sulfate to the extracted solution of ethyl acetate, concentration was performed to be dryness under a reduced pressure, whereby a crude purified substance containing the objective compound was obtained.

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